WHAT'S NEW

- New Bellco Biotechnology
- New MicroPlate Sealing Films
- New Multi-MicroPlate Genie
- Qorpak Glassware
- Qorpak Lab Coats

TRADE SHOWS

- Pittcon
  Orlando, FL
  March 13-16, 2006
  Booth #952
- American Association for Cancer Research (AACR)
  Washington, DC
  April 3-5, 2006
  Booth #320

DISRUPTOR BEADS™ — Cell Disruption Using Glass Bead Media

OVERVIEW

Spherical lead free soda lime glass beads are commonly used for mechanical disruption of many yeast, bacterial and soil samples. Glass beads of a pre-determined size and volume are placed in a 1.5ml or 2.0ml microtube along with a pre-determined sample amount. The closed tube is then shaken vigorously at high speed, causing collisions between the glass beads and sample material. Scientific Industries' Disruptor Genie™ and TurboMix™ attachment for the Vortex-Genie® 2 or Vortex-Genie® 2T are excellent choices for this process as they both simultaneously agitate and vortex at high speed, dramatically increasing cell or sample disruption. Each can hold up to twelve 1.5 ml or 2.0 ml microtubes at once. The disrupted cells may be removed after shaking for downstream processing.

Scientific Industries' Disruptor Beads are packaged as 375g (8 fl. oz.) bottles in two sizes:

0.1 mm diameter beads (Catalog No. SI-BG01)— For use with Bacteria
0.5 mm diameter beads (Catalog No. SI-BG05)— For use with Yeast/Fungi

CARE AND CLEANING

Pre-preparation steps for Scientific Industries' Disruptor Beads are generally unnecessary. If desired, they may be soaked in a 1:8 dilution of household bleach for 20 minutes, rinsed with copious amounts of distilled or RO water, and baked at 50 to 65° C for a minimum of 2 hours, or until completely dry. If the glass beads do not pour freely, repeat the cleaning and drying process. Disruptor Beads may also be autoclaved after proper disinfecting or cleaning.

The Disruptor Beads may be reused, if desired, after proper disinfecting or cleaning and autoclaving. Subsequent uses and excessive handling of the beads may result in the creation of fines, which could adversely

Company Philc

We understa customers depe products to functional, reli lasting. Frankly fanatically maintaining the Genie™ brand. aggressively e: family with u laboratory de understand that usefulness and device, there commitment to rugged, long device. It's wha means, and we that way.

If it says Gen th

1-888-I

affect cell disruption efficiency. As such, it is not recommended to frequently reuse Disruptor Beads.

Disruptor Beads may be stored at room temperature or frozen in an airtight container prior to use. In addition, the Disruptor Genie and TurboMix attachment for the Vortex-Genie 2 and Vortex-Genie 2T may be used in cold rooms.

SAMPLE APPLICATION METHODS

NOTE: DETAILED DIRECTIONS FOR USE WILL DIFFER DEPENDING ON THE INDIVIDUAL PROTOCOL USED OR THE OUTCOME DESIRED. THE SAMPLE METHODS BELOW ARE EXAMPLES ONLY.

Bacteria Disruption:

Disruptor Beads, 0.1 mm diameter, are recommended for disruption of bacterial samples. A typical sample ratio would be 50% Disruptor Beads to 50% bacterial suspension by volume. This ratio may be adjusted as necessary. Allow head space (~20%) within the microtube to facilitate disruption action. It is recommended that beads and bacterial suspension be chilled prior to disrupting in order to offset any temperature rise within the microtube. Disruption at room temperature using chilled materials for 3 to 5 minutes at highest speed should be sufficient to recover 85% of the bacterial RNA. Disruption can be performed in a cold room as well. Samples should not be run for longer than 10 minutes consecutively to avoid any temperature rise.

Yeast/Fungi Disruption:

Disruptor Beads, 0.5 mm diameter, are recommended for disruption of yeast or fungi samples. A typical sample ratio would be 50% Disruptor Beads to 50% of yeast or fungus suspension by volume. This ratio may be adjusted as necessary. Allow head space (~20%) within the microtube to facilitate disruption action. It is recommended that beads and yeast or fungus suspension be chilled prior to disrupting in order to offset any temperature rise within the microtube. Yeast cells and fungi are generally more difficult to shear than bacterial cells, so increased disruption times may be necessary. Disruption in a cold room with chilled materials for 5 to 7 minutes at highest speed should be sufficient to disrupt the cell sample. Samples should not be run for longer than 10 minutes consecutively to avoid any temperature rise.

Soil Sample Disruption:

Either size of Disruptor Beads can be used for soil samples. A typical sample ratio would be 50% Disruptor Beads to 50% soil sample suspension by volume. Allow head space (~20%) within the microtube to facilitate disruption action. Samples should not be run for longer than 10 minutes consecutively to avoid any temperature rise.

Home NewProducts Vortex-Genie® 1 Vortex-Genie® 2 Vortex-Genie® 2T Disruptor Genie® Roto-Shake Genie® Envir
BioReactor Genie™ BagRotator MagStir Genie® Disruptor Beads™ MicroPlate Genie™ MultiMagStir Genie™ Multi-Microf
Qorpak Glassware Qorpak Lab Coats Bellco Biotechnology Distributors USA/CANADA Distributors International Liter
Warranty Registration FAQ's Manuals Applications Tech Support Trade Shows Site Map About Us Financial Info Contact Us

sample at acceptable temperatures. Smaller volume shaking bead mills usually use 2 ml disposable vials. These vials have surface area to volume ratios high enough to achieve adequate heat dissipation. A popular strategy for cooling with shaking-type bead mills is to disrupt for one minute, then cool the vial in ice/water for a minute , cycling thus for the full time duration required for disruption. Temperature control is not a concern when disrupting cells or tissue in nucleic acid extraction media. Six commercial devices are currently available: the Mini-BeadBeater (BioSpec Products, Bartlesville, OK), the Micro-Dismembrator II (B. Braun Biotech, Allentown, PA), the Retsch Mixer MM 300(F. Kurt Retsch GmbH, Haan, Germany), the FastPrep Machine (Q-Biogene, Carlsbad, CA), the 2000 Geno/Grinder (SPEX CertiPrep, Metuchen, NJ), and the MagNA Lyser (Roche Diagnostics, Penzberg, Germany). While there are noticeable performance differences between these machines when disrupting especially tough cells or tissue, disruption takes about 1-5 minutes and yields are generally high. Other shaking devices or vortexers commonly found in the laboratory do not deliver enough shaking energy to get good cell disruption in a reasonable period of time. BioSpec Products manufactures three high energy shaking machines, one which holds a single 2 mL screw-cap microvial, another capable of disrupting eight 2 ml samples or five 7ml samples at a time and a third, high through-put machine capable of processing up to 48 microvials or, using deep well microplates, up to 192 samples. The price of shaking bead mills range from $600 to $9000.

Rotor-type Bead Mills. Larger capacity laboratory bead mill cell disrupters agitate the beads with a rotor rather than by shaking. Equipped with efficient cooling jackets, larger sample volumes can be processed without overheating. The most widely used is the Bead-Beater (BioSpec Products, Bartlesville, OK). This unit will disrupt about 250 mL or, with smaller chamber attachments, 50 or 15 mL batches of cell suspension in 3-5 minutes. Cell concentrations as high as forty percent (wet wt) can be used. VirTis Company (Gardiner, NY) offers an attachment for its line of high speed rotary homogenizers which efficiently agitate glass beads in a special test-tube or fluted flask. Complete homogenizer units cost about $500 and $800, respectively.

While the above small volume cell disrupters are used mostly for the disruption of microorganisms, they can also be used to homogenize and extract plant and animal tissue. This newer application is suitable for both soft tissue and tough or fibrous samples such as skin, tendon or leaves. Extraction yields of nucleic acids, viruses, receptor complexes and intracellular organelles are often superior to that of other methods. For nucleic acid isolation, consider disrupting the cells directly in the nucleic extraction solution (phenol, guanidinium SCN, etc). Nuclease concerns will be eliminated and yields enhanced. And, if PCR techniques are being used, shaking-type bead mills using disposable micro-vials and beads will totally eliminate cross contamination concerns between samples. Selective homogenization is sometimes possible using different bead sizes or speeds of bead agitation. For example, it is possible to selectively disrupt only the epidermal layer of whole leaves or to obtain intact subcellular organelles by using larger beads, smaller charges of beads and/or shorter disruption times.

ROTOR-STATOR HOMOGENIZERS (also called colloid mills or Willems homogenizers) are well suited for plant and animal tissue and generally outperform cutting-blade type Benders. Compared to a blender, foaming, swirling and aeration are minimized and smaller sample volumes are accommodated. Most tissues are quickly and thoroughly homogenized with the apparatus. The cellular material is drawn into the apparatus by a rotor sited within a static tube or stator. The material is then centrifugally thrown outward to exit through slots or holes on the tip of the stator. Because the rotor is turning at very high speed, the tissue is rapidly reduced in size by a combination of turbulence and scissor-like mechanical shearing occurring within the gap of the rotor and stator. Since most rotor-stator

homogenizers have an open configuration, the product is repeatedly recycled. The process is quite fast and, depending upon the toughness of the tissue sample, desired results are usually be obtained in 10-60 seconds. For the recovery of intracellular organelles or receptor site complexes, shorter times and/or reduced rotor speeds are used. The sample size prior to processing with the homogenizer must be small enough to be drawn inside the hole at the tip of the stator. Therefore, samples often must be pre-chopped or - fragmented with a scissors, single-edge razor blade or cryopulverizer (a device that quickly powders tissue at liquid nitrogen temperatures - see below). Unlike many other types of cell disrupters, rotor-stators homogenizers generate negligible heat during operation.

Most laboratory rotor-stator homogenizers are top driven with a compact, high speed electric motor which turns at 8,000 to 60,000 rpm. The size of the rotor-stator probe (also called the generator) can vary from the diameter of a drinking straw for 0.5-50 mL sample volumes to much larger units handling 10 liters or more. There is an important relationship between rotor speed and stator diameter. In principle, the top rotor speed of the homogenizer should double for each halving of the rotor diameter. It is not rpm per se but the tip velocity of the rotor that is the important operating parameter. Ten to twenty meters per second (2000 to 4000 fpm) are acceptable tip speeds for tissue disruption. Unfortunately, some of the smaller-sized commercial rotor-stator homogenizers do not meet this standard. Other factors such as rotor-stator design (there are many), materials used in its construction and ease of cleaning are also important factors to consider in selecting a rotor-stator homogenizer. Some manufactures are BioSpec Products (Bartlesville, OK), Brinkmann Instruments (Westbury, NY), Charles Ross & Son Company (Hauppauge, NY), Craven Laboratories (Austin, TX), IKA Works (Cincinnati, OH), Omni International (Gainsville, VA), Pro Scientific (Monroe, CT), Silverson Machines (Bay Village, OH), and VirTis Company (Gardiner, NY). The cost of complete units (motor plus rotor-stator head or generator) range from $600 to $5000.

Most laboratory sized homogenizers function properly only with liquid samples in the low to medium viscosity range (<10,000 cps). The speed and efficiency of homogenization is greatly degraded by using too small a unit, and the volume range over which a given homogenizer rotor-stator size will function efficiently is only about ten fold. Foaming and aerosols can be a problem with rotor-stator homogenizers. Keeping the tip of the homogenizer well submerged in the media and the use of properly sized vessels helps with the first problem. Square shaped homogenization vessels give better results than round vessels and it is also beneficial to hold the immersed tip off center. Aerosols can be minimized by using properly covered vessels (VirTis, Brinkmann and Omni). Even though a number of the laboratory rotor-stator homogenizers use fully enclosed motors, none of them are truly explosion-proof. Therefore, due caution should be followed when using flammable organic solvents by conducting the homogenization in a well ventilated hood.

Bottom-driven laboratory rotor-stator homogenizers are a new entry to the laboratory. The rotor-stator assembly is usually placed within a sealed chamber or container, fits blender motor bases and have working volumes of 100-1000 mL. They costs about $250 - $400 and are available from BioSpec Products (Bartlesville, OK) and Eberbach Corporation (Ann Arbor, MI).

Closely related homogenizers, called dispersers, are used for preparing large volumes of crude plant and animal aqueous extract. Operating like a household garbage disposal unit, the rotor-stator mechanism quickly homogenizes and liquefies kilogram quantities of biomass. The sample is suspended in one or more liters of media, loaded into a top reservoir and homogenized either in a continuous or batch mode. Costing $600 to $7000, two manufacturers are BioSpec

Products and IKA Works.

BLADE HOMOGENIZERS. Although less efficient than rotor-stator homogenizers and aeration and foaming can be a problem, blade homogenizers (commonly called blenders) have been used for many years to produce fine brie and extracts from plant and animal tissue. Blenders are not suitable for disruption of microorganisms. In this class of homogenizer a set of stainless steel cutting blades rotate at speeds of 6,000-50,000 rpm inside a glass, plastic or stainless steel container. The blades are either bottom- or top-driven. Some of the higher speed homogenizers can reduce tissue samples to a consistent particulate size with distributions as small as 4 microns, as determined by flow cytometric analysis. After blending, some plant tissue homogenates undergo enzymatic browning - a oxidation and cross-linking process which can complicate subsequent separation procedures. Enzymatic browning is minimized by carrying out the extraction in the absence of oxygen or in the presence of oxygen scavenging thiol compounds such as mercaptoethanol. Sometimes, addition of polyethylene imine, metal chelators, or certain detergents such as Triton X-100 or Tween 80 also help.

When using a blender, use caution when blending with flammable solvents such as alcohol or acetone or when homogenizing diseased tissues. Blenders use brush motors to achieve their high speeds and, therefore, spark during operation. Also aerosols are readily formed while blending. Use a sealed blender container and operate it in a well ventilated hood. Blade homogenizers can process liquid sample sizes from 2 mL to one gallon. Accessories for blenders include cooling jackets for temperature control, closed containers to minimize aerosol formation and entrapment of air, special vessels made of stainless steel, semi-micro containers and even insulated vessels for use with cryogenic solvents (see Freeze fracturing). Manufactures of a scientific line of blenders include British Medical Enterprises (London, England), ESGE (Basel, Switzerland), Hamilton Beach Commercial (Washington, NC), Omni International (Waterbury, CT), Professional Diagnostic (Edmonton, Alberta Canada), The VirTis Company (Gardiner, NY) and Waring Products Division (New Hartford, CT). Accessory vessels for Hamilton-Beach brand blenders are manufactured by BioSpec Products (Bartlesville, OK) and for Waring brand blenders by Eberbach Corporation (Ann Arbor, MI). Prices for blade homogenizers range from about $100 to $2000.

FREEZE-FRACTURING. Both microbial pastes and plant and animal tissue can be frozen in liquid nitrogen and then ground with a common mortar and pestle at the same low temperature. Presumably the hard frozen cells are fractured under the mortar because of their brittle nature. Also, ice crystals at these low temperatures may act as an abrasive.

A freeze-fracturing device called the Bessman tissue pulverizer is useful for fragmenting 10 mg to 10 g quantities of fibrous tissue such as skin or cartilage to the size of grains of salt. This material is then easily homogenized by other methods. Looking somewhat like a tablet press, the pulverizer consists of a hole machined into a stainless steel base into which fits a piston. The base and piston are pre-cooled to liquid nitrogen temperatures. Ten mg to ten grams of hard frozen animal or plant tissue is placed in the hole. The piston is placed in the hole and given a sharp blow with a hammer. The resulting frozen, powder-like material can be further processed by Pestle and Tube, Bead Mill or Rotor-stator homogenizers. Manufactured by BioSpec Products (Bartlesville, OK) and by Spectrum Medical Industries (Carson, CA), the pulverizers come in several sizes and cost $200 to $400. In larger capacity models, BioSpec Products (Bartlesville, OK) has incorporated a built-in, spring loaded hammer looking much like a staple gun. This same company also makes a small screw press designed to pulverize 50-500 mg of hard frozen tissue. This pulverization tool works slower than the Bessman device (perhaps a minute rather than a few seconds per sample), but it is well suited for

fresh bone and other hard tissue.

GRINDERS. Grinding biological material in a mortar or tube with fine sand, alumina or glass powder is roughly the equivalent of bead-milling (see above). The method works reasonably well with all types of biomass but is strictly small scale and is labor intensive. Cell pastes or solid mass with a minimum volume of buffer are mixed with 0.5-1 volume of grinding media and ground with a mortar and pestle. Disruption efficiency is poor if lower cell densities or smaller charges of grinding media are used. Glass powder, having a high surface area, may adsorb significant amounts of charged biomolecules such as nucleic acids and proteins.

PESTLE AND TUBE HOMOGENIZERS (also called tissue grinders) are used to disrupt animal tissue. While variations of the pestle and tube homogenizer have names like Potter, Potter-Elvehjem, Dounce, and Ten Broeck, as a group they consist of test-tubes made of glass, inert plastic or stainless steel into which is inserted a tight-fitting pestle (clearance about 0.1-0.2 mm) made of like material. The walls of the test-tube and pestle can be smooth or have a ground finish. Most tissues must be cut or chopped into small pieces (1-5 mm) with scissors or a single-edge razor blade before being suspended in a 4-10 fold volume excess of medium in the test-tube. The pestle is manually worked to the bottom of the tube, thus tearing and fragmenting tissue as it is forced to pass between the sides of the pestle and the wall of the tube. The grinding action occurs again as the pestle is withdrawn. Five to thirty repetitions of this low shear method homogenizes the tissue. Rotation of the pestle at about 500-1000 rpm with an electric motor while the test-tube is manually raised and lowered speeds up the process. While pestle and tube homogenization is simple and the equipment used is usually inexpensive, it is both labor intensive and, in the case of fragile glass homogenizers, potentially dangerous. Even so, this homogenizer will continue to be popular because of its extremely gentle action. Often it is the method of choice for the preparation of small quantities of subcellular organelles from soft animal tissues such as brain or liver. Microorganisms cannot be disrupted with pestle homogenizers. Commercially available glass or plastic pestle homogenizers with batch capacities of 0.1-50 mL generally cost $15-$100 and are available from many manufacturers including Ace Glass (Vineland, NJ), Bellco Glass (Vineland, NJ), BioSpec Products (Bartlesville, OK), Kontes (Vineland, NJ), Thomas Scientific (Swedesboro, NJ), Tri-R Instruments (Rockville Center, NY), Sage Products (Crystal Lake, IL) and Wheaton Industries (Milville, NJ). Disposable, plastic pestles which fit into microcentrifuge tubes are available from Kontes. They also offer a small, hand-held motor unit to drive the pestle. While precision stainless steel tissue grinders are more expensive ($200 - $250, BioSpec Products and Wheaton), they are efficiently cooled and tolerate vigorous homogenization without risk of breakage. A 'Rolls-Royce' homogenizer costing about $3000 has a variable speed motor, cooling jacket, and hand- operated lever to rise and lower the pestle (B. Braun Biotech Bethlehem, PA). A continuous pestle homogenizer is available from Yamato USA (Northbrook, IL). Grooves machined on the upper one-third of the pestle catch and guide tissue through the close tolerance region of the lower two-thirds of the cylinder pestle. The resultant homogenate exits from the bottom of the cylinder. Recycling is usually necessary. The machine comes in two sizes and costs $2000-$3000.

MEAT MINCER AND SOLIDS PRESS. The household meat grinder or mincer has been used for many years for the preparation of animal tissue extracts. Tissue is mechanically pressed through holes in a metal sieve plate while rotating blades slowly sweep across the face of the plate cutting the meat in 0.3-0.5 mm fragments. While it is not an effective way to disrupt cells per se , it is useful as a preliminary step for complete homogenization using other physical or chemical methods. Meat grinders cut flexible tissue like muscle better if the tissue is processed slightly frozen.

For small tissue samples, BioSpec Products (Bartlesville, OK) manufacture hand operated screw presses for the preparation of tissue extracts as does EDCO Scientific (Chapel Hill, NC). Capable of considerable force, sample sizes from 0.1 grams up to 50 grams of soft tissue are pushed through sieve plates having 0.5 to 3 mm holes, much like the action of a kitchen garlic press. Hard or fibrous tissues like tendon, skin, leaves and seeds will not pass through the press. The units cost from $25 to $400. Fred S. Carver (Wabash, IN) has a compact hydraulic laboratory tissue press for the extraction of intracellular liquids and oils for about $1600. Designed for plant leaves, Bioreba (Chapel Hill, NC) makes a hand-held grinder consisting of an circular array of steel balls which crush a few leaves inside a mylar plastic bag. The grinder and bags cost about $200.

ULTRASONIC DISINTEGRATORS are widely used to disrupt cells. These devises generate intense sonic pressure waves in liquid media. Under the right conditions, the pressure waves cause formation of microbubbles which grown and collapse violently. Called cavitation, the implosion generates a shock wave with enough energy to break cell membranes and even break covalent bonds.

Modern ultrasonic processors use piezoelectric generators made of lead zirconate titanate crystals. The vibrations are transmitted down a titanium metal horn or probe tuned to make the processor unit resonate at 15-25 kHz. The rated power output of ultrasonic processors vary from 10 to 375 Watts. What really counts is the power density at the probe tip. Higher output power is required to sustain good performance in large sized probes. For cell disruption, probe densities should be at least 100 W/cm^2 and the larger the better for tip amplitude (typical range: 30-250 microns). Some manufacturers of ultrasonic disintegrators are Artek Systems (Farmington, NY), BioSpec Products (Bartlesville, OK), Branson Sonic Power Company (Danbury, CT), RIA Research Corp. (Hauppauge, NY), Sonic Systems (Newton, PA), Ultrasonic Power Corporation (Freeport, IL) and VirTis Company (Gardiner, NY).

Ultrasonic disintegrators generate considerable heat during processing. For this reason the sample should be kept ice cold. For microorganisms the addition of 0.1 - 0.5 mm diameter glass beads in a ratio of one volume beads to two volumes liquid is recommended, although this modification will eventually erode the sonicator tip. Tough tissues like skin or tendon should be macerated first in a tissue press, grinder or pulverized in liquid nitrogen (see details above). Use small vessels during ultrasonic treatment and place the probe tip deep enough in the sample to avoid foaming. Finally, one should be aware that free radicals can be generated during sonication and that these radicals react with most biomolecules. Damage by oxidative free radicals can be minimized by flushing the solution with nitrogen and/or including scavengers like cysteine, dithiothreitol or other -SH compounds in the media.

OTHER CELL DISRUPTION APPARATUS OR TECHNIQUES not covered in this review but discussed by the author in the book, *Purification and Analysis of Recombinant Proteins, Seetharam and Sharma, editors, published by Marcel Dekker, Inc., 1991* are High-Pressure Homogenizers, Autolysis, Enzymatic lysis, Dehydration, Chemical lysis, Solvent lysis and Programmed self-destruction.

Last modified: 11/29/05.

FosmidMAX™ DNA Purification Kit

*Cat. No. **FMAX046***

The **FosmidMAX™ DNA Purification Kit** was developed for easy, reliable isolation of high-quality fosmid DNA. The scalable protocol is based on a modified alkaline-lysis procedure that can be used with 1.5–100 ml of culture. Consistent yields of up to 0.6, 15 or 25 µg of fosmid DNA are obtained from 1.5, 40 or 100 ml cultures of a single-copy fosmid, respectively. Up to 4 µg of fosmid DNA can be isolated from 1.5 ml of an induced CopyControl™ fosmid clone (see below). Selective precipitation steps and the incorporation of EPICENTRE's RiboShredder™ RNase Blend effectively remove contaminants that degrade DNA and interfere with downstream applications. There is no need for columns, resins or organic extractions. The exceptionally pure fosmid DNA can be used for many applications, including sequencing, fingerprinting, PCR and preparation of shotgun libraries. The FosmidMAX Kit can also be used to isolate cosmid DNA.

EPICENTRE also offers the CopyControl Fosmid Library Production Kit.[1] This kit utilizes a strategy of cloning blunt-ended DNA fragments, generated by random shearing of DNA, to produce complete and unbiased genomic libraries. Greater than 10^6 clones are produced in a single experiment. The unique CopyControl cloning technology enables the user to grow the clones at single copy to ensure insert stability and cloning of toxic gene products and then induce the clones to high-copy number for high yields of DNA.

Product Specifications

Storage: Store the RiboShredder RNase Blend at -20°C in a freezer without a defrost cycle. Store the remainder of the kit components at room temperature.

FosmidMAX™ DNA Purification Kit Contents

The FosmidMAX™ DNA Purification Kit contains sufficient reagents to perform 150 x 1.5 ml, 10 x 40 ml or 5 x 100 ml purifications.

FosmidMAX™ Solution 1	30 ml
FosmidMAX™ Solution 2*	60 ml
FosmidMAX™ Solution 3	45 ml
FosmidMAX™ Solution 4	38 ml
RiboShredder™ RNase Blend	200 µl
TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)	42 ml

** FosmidMAX™ Solution 2 may form a precipitate during storage. If this occurs, heat the bottle at 37°C until the precipitate dissolves. Mix thoroughly and cool to room temperature.*

Quality Control: The FosmidMAX DNA Purification Kit is function-tested by purifying a single-copy fosmid clone. DNA quality and yield are assayed by gel electrophoresis, fluorimetry and restriction enzyme digestion.

Related Products: The following products are also available:

- CopyControl™ Fosmid Library Production Kit
- EpiFOS™ Fosmid Library Production Kit
- pWEB™ Cosmid Cloning Kit
- CopyControl™ BAC Cloning Kits
- Fast-Link™ DNA Ligation Kits
- End-It™ DNA End-Repair Kit

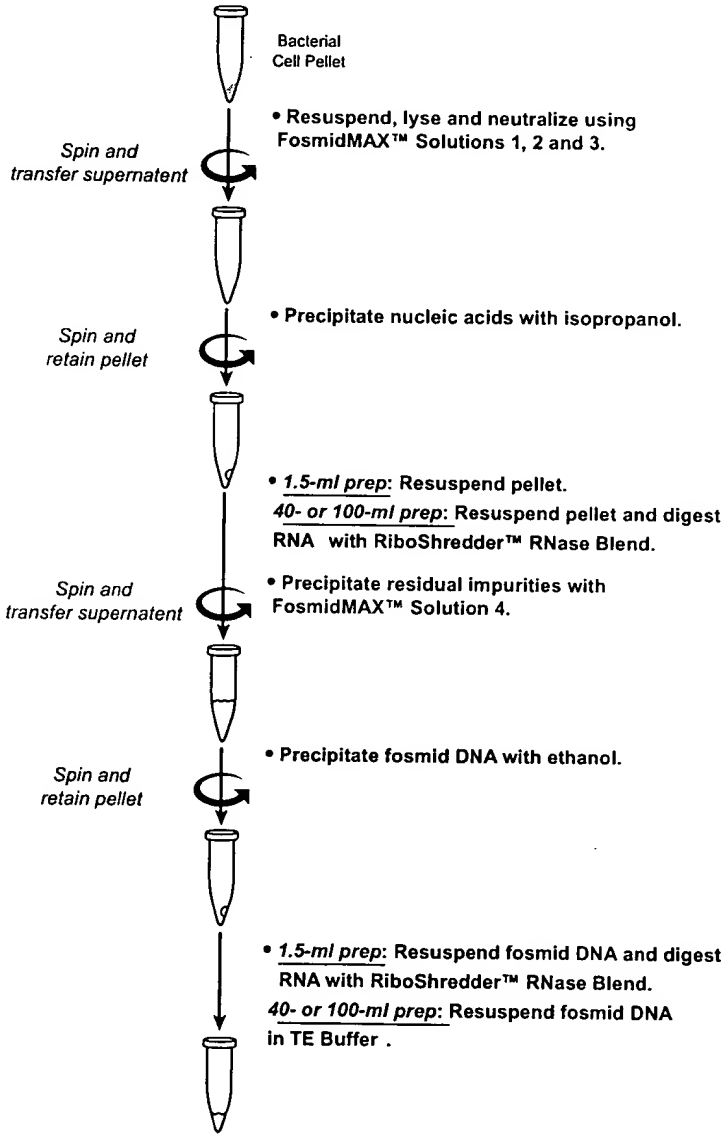
References:

1. *EPICENTRE Forum* (2002) **9** (1), 3.

-continued

Figure 1. An overview of the FosmidMAX™ DNA Purification Kit protocol.

The recommended protocol for 1.5-purifications is shown here and described in Section A. Yields are maximized by including the RNA digestion step at the end of the protocol. This enables the RNA to act as a carrier which facilitates DNA precipitation and visualizing the sample pellet. In our experience, downstream applications, like fingerprinting, sequencing and shotgun library construction, are not inhibited by the small amount of residual free nucleotides or the RiboShredder Blend. However, the alternative protocol described in Section B includes an RNA digestion step earlier in the purification process so these residual contaminants are eliminated.



General Considerations

1. **Optimal cell density**: Harvest cells at an OD_{600} of 3 to 4 to maximize yields of fosmid DNA. Growing cells for more than 16 hours is not recommended.

2. **Avoid shearing**: Fosmid DNA, because of its large size, is prone to shearing. Do **NOT** vortex, shake or pipet the cells after adding FosmidMAX Solution 2 and 3 during the lysis and neutralization steps. The lysis reaction should not exceed 5 minutes. Mix with gentle inversion and use a wide-orifice pipet where noted in the protocol.

3. **Proper storage conditions:** Store fosmid DNA at -20°C in small aliquots so that repeated freeze-thaw cycles are avoided.

Fosmid DNA Purification Protocols

A. Purification from 1.5 ml of Culture

Note: The protocol given below maximizes yields by including the RNA digestion step at the end of the procedure. This enables the RNA to act as a carrier which facilitates DNA precipitation and visualizing the sample pellet. In our experience, downstream applications, like fingerprinting, sequencing and shotgun library construction, are not inhibited by the small amount of residual free nucleotides or the RiboShredder Blend. However, the alternative protocol described in Section B includes an RNA digestion step earlier in the purification process so these residual contaminants are eliminated.

Growing the culture: Prepare 2 ml of LB medium containing the appropriate antibiotic in a 14 ml snap cap culture tube. Inoculate with an isolated colony from a freshly streaked plate and shake (~250 rpm) at 37°C for 12-16 hours. The culture should be grown to an OD_{600} of 3 to 4.

Prior to starting: Chill FosmidMAX Solutions 1, 3 and 4 on ice.
Dilute RiboShredder RNase Blend 1:4 in TE Buffer. Keep on ice until needed.

1. Transfer 1.5 ml of the overnight culture to a 1.7 ml microcentrifuge tube. Pellet the cells by centrifuging at 15,000 x g or maximum speed for 1-3 minutes. Discard the supernatant.

2. Add 200 µl of chilled FosmidMAX Solution 1 to the pellet. Vortex vigorously to completely resuspend the pellet. Make sure the pellet is completely resuspended before proceeding.

3. Add 400 µl of FosmidMAX Solution 2. Mix by inverting the tube 2-3 times very gently. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate. *Note: The lysis reaction should not exceed 5 minutes.*

4. Add 300 µl of chilled FosmidMAX Solution 3. Mix by inverting the tube 2-3 times very gently. A white precipitate will form in the tube. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate.

5. Incubate on ice for 15 minutes.

6. Centrifuge at 15,000 x g or maximum speed for 15 minutes at 4°C to pellet cellular debris.

7. Transfer the supernatant to a microcentrifuge tube. *Note: To avoid pipetting the white precipitate floating on the surface of the supernatant, place the pipet tip below the meniscus and aspirate slowly, without disturbing the pellet.*

8. Add 540 µl or 0.6 volumes of room temperature isopropanol to the recovered supernatant. Mix thoroughly by inverting the tube 4-6 times.

9. Precipitate the nucleic acids by centrifugation at 15,000 x g or maximum speed for 15 minutes at 4°C. Carefully decant the isopropanol. Centrifuge briefly and pipet off any excess isopropanol without disrupting the pellet.

10. Air-dry the pellet at room temperature for 3-5 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.

11. Resuspend the pellet in 250 µl of TE Buffer by tapping and swirling the tube. Make sure the DNA is completely dissolved before proceeding.

12. Add 250 µl of chilled FosmidMAX Solution 4 to the tube. Mix thoroughly by tapping the tube and incubate on ice for 15 minutes.

13. Centrifuge the tube at 15,000 x g or maximum speed for 15 minutes at 4°C. Carefully transfer the supernatant to a microcentrifuge tube without disrupting the pellet.

14. Add 1 ml of absolute ethanol (200 proof) to the recovered supernatant. Mix gently by inverting the tube 4-6 times.

15. Precipitate the DNA by centrifugation at 15,000 x g or maximum speed for 15 minutes at 4°C. Carefully pipette off the ethanol without disrupting the pellet. Centrifuge briefly and pipette off any residual ethanol.

16. Air-dry the pellet at room temperature for 3-5 minutes. Do **NOT** over-dry or it will be difficult to re-suspend the pellet.

17. Add 25 µl of TE Buffer to the tube (sterile deionized water or Tris-buffer can also be used) by tapping the tube and leave at room temperature for 10 minutes.

18. Add 1 µl of diluted RiboShredder RNase Blend to the tube and incubate at 37°C for 30 minutes.

19. Quanitate the yield of fosmid DNA by fluorimetry using a DNA specific dye (e.g. PicoGreen® or bis-benzimide [Hoechst dye 33258]) or by agarose gel electrophoresis.

20. Store the fosmid DNA at -20°C in small aliquots so that repeated freeze-thaw cycles are avoided.

B. Alternative Protocol for Purification from 1.5 ml of Culture

Growing the culture: Prepare 2 ml of LB medium containing the appropriate antibiotic in a 14 ml snap cap culture tube. Inoculate with an isolated colony from a freshly streaked plate and shake (~250 rpm) at 37°C for 12-16 hours. The culture should be grown to an OD_{600} of 3 to 4.

Prior to starting: Chill FosmidMAX Solutions 1, 3 and 4 on ice.

Steps 1-11 are as described in Section A (page 3).

12. Add 1 µl of undiluted RiboShredder RNase Blend to the tube and incubate at 37°C for 30 minutes.

13. Add 250 µl of chilled FosmidMAX Solution 4. Mix gently by tapping the tube and incubate on ice for 15 minutes.

14. Centrifuge the tube at 15,000 x g or maximum speed for 15 minutes at 4°C. Carefully transfer the supernatant to a microcentrifuge tube without disrupting the pellet. *Note: Since the pellet may not be visible after centrifugation, keep the orientation of tubes in the centrifuge consistent so the location of the pellet is always known.*

15. Add 1 ml of absolute ethanol (200 proof) to the recovered supernatant. Mix gently by inverting the tube 4-6 times.

16. Precipitate the DNA by centrifugation at 15,000 x g or maximum speed for 15 minutes at 4°C. Carefully pipette off the ethanol without disrupting the pellet. Centrifuge briefly and pipette off any residual ethanol.

17. Air-dry the pellet at room temperature for 3-5 minutes. Do **NOT** over-dry or it will be difficult to re-suspend the pellet.

18. Resuspend the pellet in 25 µl of TE Buffer (sterile deionized water or Tris-buffer can also be used) by tapping and swirling the tube.

19. Quanitate the yield of fosmid DNA by fluorimetry using a DNA specific dye (e.g. PicoGreen® or bis-benzimide [Hoechst dye 33258]) or by agarose gel electrophoresis.

20. Store the fosmid DNA at -20°C in small aliquots so that repeated freeze-thaw cycles are avoided.

C. Purification from 40 ml of Culture

Growing the culture: Prepare 50 ml of LB medium containing the appropriate antibiotic in a 250 ml flask. Inoculate with an isolated colony from a freshly streaked plate and shake vigorously (~250 rpm) at 37°C for 14-16 hours. The culture should be grown to an OD_{600} of 3 to 4.

Prior to starting: Chill FosmidMAX Solutions 1, 3 and 4 on ice.

1. Transfer 40 ml of the overnight culture to a 40 ml Oakridge-style centrifuge tube. Pellet the cells by centrifuging at 5,000 x g for 8 minutes at 4°C. Discard the supernatant.

2. Add 3 ml of chilled FosmidMAX Solution 1 to the pellet. Vortex vigorously to completely resuspend the pellet. Make sure the pellet is completely resuspended before proceeding.

3. Add 6 ml of FosmidMAX Solution 2. Mix by inverting the tube 2-3 times very gently. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate. *Note: The lysis reaction should not exceed 5 minutes.*

4. Add 4.5 ml of chilled FosmidMAX Solution 3. Mix by inverting the tube 2-3 times very gently. A white precipitate will form in the tube. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate.

5. Incubate on ice for 15 minutes.

6. Centrifuge at 15,000 x g for 15 minutes at 4°C to pellet cellular debris.

7. Transfer the supernatant to a 40 ml Oakridge-style centrifuge tube using a 10 ml pipet. *Note: To avoid pipetting the white precipitate floating on the surface of the supernatant, place the pipet tip below the meniscus and aspirate slowly, without disturbing the pellet.*

8. Add 8.1 ml or 0.6 volumes of room temperature isopropanol to the recovered supernatant. Mix thoroughly by inverting the tube 4-6 times.

9. Precipitate the nucleic acids by centrifugation at 15,000 x g for 15 minutes at 4°C. Carefully decant the isopropanol. Centrifuge briefly and pipet off any excess isopropanol without disrupting the pellet.

10. Air-dry the pellet at room temperature for 3-5 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.

11. Resuspend the pellet in 500 µl of TE Buffer by tapping and swirling the tube. Make sure the DNA is completely dissolved before proceeding.

12. Add 18 µl of RiboShredder RNase Blend to the tube and incubate at 37°C for 30 minutes. Add an additional 500 µl of TE Buffer and mix by tapping the tube.

13. Add 1 ml of chilled FosmidMAX Solution 4. Mix gently by tapping the tube and incubate on ice for 15 minutes.

14. Centrifuge the tube at 15,000 x g for 15 minutes at 4°C. Carefully transfer the supernatant to a 40 ml Oakridge-style centrifuge tube without disrupting the pellet. *Note: Since the pellet may not be visible after centrifugation, keep the orientation of tubes in the centrifuge consistent so the location of the pellet is always known.*

15. Add 4 ml of absolute ethanol (200 proof) to the recovered supernatant in each tube. Mix gently by inverting the tube 4-6 times.

16. Precipitate the DNA by centrifugation at 15,000 x g for 15 minutes at 4°C. Carefully decant the ethanol. Centrifuge briefly and pipette off any residual ethanol without disrupting the pellet. *Note: Since the pellet may not be visible after centrifugation, keep the orientation of tubes in the centrifuge consistent so the location of the pellet is always known.*

17. Air-dry the pellet at room temperature for 5-7 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.

18. Add 200 µl of TE Buffer to each tube (sterile deionized water or Tris-buffer can also be used). Resuspend the pellet by tapping the tube and leave the DNA overnight at 4°C to dissolve completely.

19. Quanitate the yield of fosmid DNA by fluorimetry using a DNA specific dye (e.g. PicoGreen® or bisbenzimide [Hoechst dye 33258]) or by agarose gel electrophoresis.

D. Purification from 100 ml of Culture

Growing the culture: Prepare 100 ml of LB medium containing the appropriate antibiotic in a 500 ml flask. Inoculate with an isolated colony from a freshly streaked plate and shake vigorously (~250 rpm) at 37°C for 14-16 hours. The culture should be grown to an OD_{600} of 3 to 4.

Prior to starting: Chill FosmidMAX Solutions 1, 3 and 4 on ice.

1. Transfer 100 ml of the overnight culture to a 250 ml centrifuge bottle. Pellet the cells by centrifuging at 5,000 x g for 8 minutes at 4°C. Discard the supernatant.

2. Add 6 ml of chilled FosmidMAX Solution 1 to the pellet. Vortex vigorously to completely resuspend the pellet. Make sure the pellet is completely resuspended before proceeding.

3. Transfer equal volumes of the cell suspension (3 ml) to two 40 ml Oakridge-style centrifuge tubes.

4. Add 6 ml of FosmidMAX Solution 2 to each tube. Mix by inverting the tube 2-3 times very gently. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate. *Note: The lysis reaction should not exceed 5 minutes.*

5. Add 4.5 ml of chilled FosmidMAX Solution 3 to each tube. Mix by inverting the tube 2-3 times very gently. A white precipitate will form in the tube. To avoid shearing the fosmid DNA do **NOT** vortex, shake or pipet the lysate.

6. Incubate on ice for 15 minutes.

7. Centrifuge at 15,000 x g for 15 minutes at 4°C to pellet cellular debris.

8. Transfer the supernatant to a 40 ml Oakridge-style centrifuge tube using a 10 ml pipet. *Note: To avoid pipetting the white precipitate floating on the surface of the supernatant, place the pipet tip below the meniscus and aspirate slowly, without disturbing the pellet.*

9. Add 8.1 ml or 0.6 volumes of room temperature isopropanol to the recovered supernatant. Mix thoroughly by inverting the tube 4-6 times.

10. Precipitate the nucleic acids by centrifugation at 15,000 x g for 15 minutes at 4°C. Carefully decant the isopropanol. Centrifuge briefly and pipet off any excess isopropanol without disrupting the pellet.

11. Air-dry the pellet at room temperature for 3-5 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.

12. Resuspend the pellet in 500 µl of TE Buffer by tapping and swirling the tube. Make sure the DNA is completely dissolved before proceeding.

13. Add 20 µl of RiboShredder RNase Blend to the tube and incubate at 37°C for 30 minutes. Add an additional 500 µl of TE Buffer and mix by tapping the tube.

14. Add 1 ml of chilled FosmidMAX Solution 4 to each tube. Mix gently by tapping the tube and incubate on ice for 15 minutes.

15. Centrifuge the tube at 15,000 x g for 15 minutes at 4°C. Carefully transfer the supernatant to a 40 ml Oakridge-style centrifuge tube without disrupting the pellet. *Note: Since the pellet may not be visible after centrifugation, keep the orientation of tubes in the centrifuge consistent so the location of the pellet is always known.*

16. Add 4 ml of absolute ethanol (200 proof) to the recovered supernatant in each tube. Mix gently by inverting the tube 4-6 times.

17. Precipitate the DNA by centrifugation at 15,000 x g for 15 minutes at 4°C. Carefully decant the ethanol. Centrifuge briefly and pipette off any residual ethanol without disrupting the pellet. *Note: Since the pellet may not be visible after centrifugation, keep the orientation of tubes in the centrifuge consistent so the location of the pellet is always known.*

18. Air-dry the pellet at room temperature for 5-7 minutes. Do **NOT** over-dry or it will be difficult to resuspend the pellet.

19. Add 200 µl of TE Buffer to each tube (sterile deionized water or Tris-buffer can also be used). Resuspend the pellet by tapping the tube and leave the DNA overnight at 4°C to dissolve completely.

20. Quanitate the yield of fosmid DNA by fluorimetry using a DNA specific dye (e.g. PicoGreen® or bisbenzimide [Hoechst dye 33258]) or by agarose gel electrophoresis.